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Carbamate substituted 2-amino-4,6-diphenylpyrimidines as adenosine receptor antagonists

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ABSTRACT

A novel series of carbamate substituted 2-amino-4,6-diphenylpyrimidines was evaluated as potential dual adenosine A_1 and A_{2A} receptor antagonists. The majority of the synthesised compounds exhibited promising dual affinities, with A_1K_i values ranging from 0.175 to 10.7 nM and $A_{2A}K_i$ values ranging from 1.58 to 451 nM. The in vivo activity illustrated for 3-(2-amino-6-phenylpyrimidin-4-yl)phenyl morpholine-4-carboxylate (**4c**) is indicative of the potential of these compounds as therapeutic agents in the treatment of Parkinson's disease, although physicochemical properties may require optimisation.

Parkinson's disease (PD) is a chronic neurodegenerative disease that affects over 1% of the world population. PD is an age related disease, and with life expectancy increasing worldwide, will continue to present a huge social and economic burden in the future.[1] Patients with PD mainly suffer from a progressive loss of motor function, but non-motor symptoms, such as cognitive impairment and depression often occur.[2] The motor symptoms of the disease can be attributed to the deterioration of dopaminergic neurons in the striatum, resulting in a significant loss of dopamine in this region.[3] There is still no cure for PD, but the dopaminergic therapies used clinically are reasonably effective in managing the symptoms during the early stages of the disease. Long-term treatment with dopaminergic therapies however, is associated with several undesirable side effects such as loss of drug efficacy, dyskinesia and depression.[4]

Adenosine is an endogenous ligand that acts as a neurotransmitter in the brain through the activation of its G-protein coupled receptors, namely the A_1, A_{2A}, A_{2B} and A_3 receptors.[5] Recently, dual targeted antagonism of adenosine A_1 and A_{2A} receptors has emerged as a promising non-dopaminergic alternative for the treatment of neurodegenerative diseases such as Parkinson's disease.[6,7] The appeal of adenosine A_{2A} receptors as a target in movement disorders is due to their distinct localisation in the striatum as well as their unique integrative action with dopamine D_2 receptors.[8] Adenosine A_{2A} receptors and dopamine D_2 receptors have a mutual antagonistic interaction, which means that antagonism of adenosine A_{2A} receptors would lead to enhanced D_2 signalling, providing a rationale for their use in the symptomatic treatment of PD.[9] The use of A_{2A} antagonists adjunctive to current dopaminergic therapy may be beneficial since the dosage of the dopaminergic drugs administered could potentially be lowered.[10] This may reduce the occurrence of dyskinesia and other side effects associated with dopaminergic drugs.[11] Furthermore, it has been suggested that A_{2A} antagonism may halt the progression of PD as preclinical evidence exists of its possible neuroprotective benefits.[12,13] Depression is a co-morbidity that often decreases quality of life in PD patients, especially in the later stages of the disease. A recent study illustrated the antidepressant effect of the known A_{2A} antagonist, KW6002, indicating an additional advantage of A_{2A} antagonism.[14]

The brain distribution of the adenosine A_1 receptor on the other hand, is more widespread than that of the A_{2A} receptor, with high levels expressed in the striatum, hippocampus and neocortex.[15] Similar to adenosine A_{2A} antagonism, antagonism of A_1 receptors have been shown to result in activation of motor function in animals,[16,17] and may thus decrease motor deficiencies experienced in PD. It has also been reported that the antagonism of the A_1 receptor may enhance cognitive ability,[18–21] and since a decline in cognition is often observed in PD patients over time,

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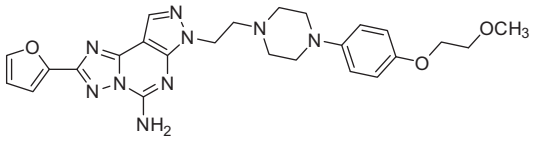
A_1 antagonism may thus be advantageous.[22] Dual antagonism of A_1 and A_{2A} receptors thus has the potential of addressing the multifactorial nature of PD symptoms with less dopaminergic side-effects than generally experienced with current therapies.

The best known adenosine receptor antagonists are the xanthines, such as caffeine and theophylline, which as a chemical class, have been reviewed extensively.[18] Several heterocyclic compounds have also progressed to clinical trials, and include compounds such as preladenant (**1**) and tozadenant (**2**).[23,24]



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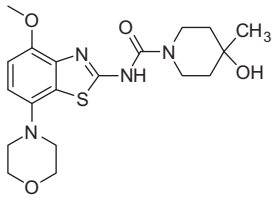

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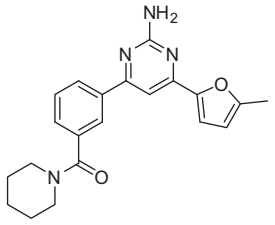
Of particular interest to our group was the fact that the 2-aminopyrimidine motif often occurred in heterocycles with adenosine A_{2A} and/or A_1 affinity.[7,25–29] Based on the aforementioned results, we set out to synthesise a small library of 2-aminopyrimidines to investigate the potential of these compounds as dual adenosine A_1 and A_{2A} antagonists. In a preliminary study reported recently, we synthesised amide derivative **3** which exhibited high dual affinity (A_1K_i = 9.54 nM; A_{2A} K_i = 6.34 nM) and in vivo activity.[30]



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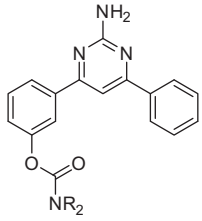

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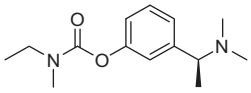

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In order to further investigate the affinities of this class of compounds for adenosine receptors, we decided to synthesise a series of carbamate substituted 2-amino-4,6-diphenylpyrimidines (**4**). The carbamate moiety is often present in therapeutic agents, such as rivastigmine (**5**), an acetylcholinesterase inhibitor, which is used in the treatment of Alzheimer's disease. This amide-ester hybrid generally displays very good chemical and proteolytic stability and may increase permeability across cellular membranes.[31] The addition of the extra oxygen in structures such as **4**, alters the position of both the carbonyl and nitrogen groups and has the potential to change the hydrogen bonding between the compound and the receptor binding site, thus changing the affinity and possibly the selectivity of these compounds in comparison with the amide derivatives (**3**). It was also decided to replace the methyl furan substituent on position 4 with a phenyl ring, as this simplified the synthesis. Gratifyingly, preliminary results indicated that this change did not alter affinity to a significant degree.

Nine carbamate substituted 2-amino-4,6-diphenylpyrimidines (Table 1) were successfully synthesised as indicated in Scheme 1. Firstly, 3-hydroxybenzaldehyde (**6**) was condensed with acetophenone (**7**), yielding chalcone (**8**), which was reacted with commercially available carbamoyl chlorides to obtain carbamates (**9a–i**).[32] Cyclisation was carried out with guanidine hydrochloride and sodium hydride in *N,N*-dimethylformamide yielding the desired 2-aminopyrimidines (**4a–i**) in low yields.[33] Initially, three equivalents of sodium hydride were used in the cyclisation step, but this resulted in cleavage of the carbamate group. This problem was overcome by reducing the number of molar equivalents of NaH used in the reaction. The structures of all synthesised compounds (Table 1) were confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry, while purity was assessed by HPLC (see Supporting Information).

Radioligand binding assays were performed to assess the binding of synthesised compounds to adenosine receptors. The radioligands used were 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) for adenosine A_1 receptors, and [^{3}H]5'-*N*-ethylcarboxamide-adenosine ([^{3}H]NECA) for adenosine A_{2A} receptors. Striata from male Sprague–Dawley rats were used as receptor source for A_{2A} binding studies, while whole brains were utilised for A_1 binding studies (Ethics number: NWU-0035-10-A5). IC_{50} values were obtained from sigmoidal-dose response curves as generated by the Prism 5 software package (GraphPad) and the K_i values were calculated from the IC_{50} values using the Cheng–Prusoff equation.[34] Results from the receptor binding studies are presented in Table 1.

The results reveal that most of these compounds have potent dual affinity for both receptor subtypes, although affinities are generally higher for the adenosine A_1 receptor compared to the adenosine A_{2A} receptor. Compounds **4a**, **4b** and **4c** are the most promising candidates for dual antagonistic activity with both A_1K_i and $A_{2A}K_i$ values below 10 nM and selectivity indices of 4.6, 0.8 and 1.3, respectively. Compounds **4f** and **4g** in particular exhibit high affinities for the adenosine A_1 receptors, with K_i values of 0.468 and 0.175 nM, respectively. As exemplified by compounds **4a**, **4b** and **4c**, substitution with six-membered saturated cyclic carbamate substituents appears to yield optimal A_{2A} receptor affinity. Interestingly, although compound **4i** with diphenyl substitution still has high affinity for the adenosine A_1 receptor (K_i = 10.7 nM), its affinity for the adenosine A_{2A} receptor is comparatively weak with a K_i value of 451 nM. The size of the carbamate